Gelatin freeze casting of biomimetic titanium alloy implants with anisotropic and gradient pore structure

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Abstract. Titanium metal and its alloys are materials commonly used for dental and orthopaedic implants. However, due to large difference in properties between the titanium metal and the natural bone, stress shielding has been observed around the surround area, resulting in bone atrophy, and thus has raised concerns of the use of this material. Ideally implant materials should possess similar properties to the surrounding tissues in order to distribute the load as the joint would naturally, while also possessing a similar porous structure to the bone to enable interaction with the surrounding material. In this paper we report the formation of aligned porous titanium alloy scaffolds with the use of unidirectional freeze casting with a temperature gradient. The resulting scaffolds had a dense bottom part with sufficient strength for loading, while the top part remaining porous in order to allow bone growth in the scaffold and fully integrating with the surrounding tissue. The anisotropic nature of the pores within the titanium alloy samples were observed *via* micro computed tomography, where a gradient structure similar to bone was observed. The compressive strength of the fabricated scaffolds was found to be up to 427 MPa when measured with the pores aligned with the applied load, depending on the pore density. This is within the range of cortical bone.

Keywords: biomaterials; bone scaffolds; freeze casting; titanium; gelatin; micro computed tomography

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1. Introduction

Bone is a ‘living’ material with remarkable mechanical properties attributed by its anisotropic and hierarchical structure (Weiner and Wagner 1998). Any material used to replace damaged or diseased bone should possess suitable biocompatibility, biofunctionality and biomechanical properties. Due to the inferior mechanical properties of ceramics (*i.e.* brittleness) and polymers (*i.e.* low mechanical strength), titanium metal and its alloys are preferred materials for orthopaedic and dental implants. However, solid titanium implants may incur stress shielding problems because of the mechanical stiffness mismatch between implant and bone, which leads to implant loosening or marginal bone loss surrounding the implant. Titanium metal and its alloys have much higher stiffness than cortical bone (110 GPa vs 20 GPa). Since bone is a dynamic tissue, its density and mechanical properties are constantly remodelled according to its mechanical loadings. Thus the stiffer metal implant would carry the majority of applied loads, leaving the bone tissue effectively unstressed, which causes bone atrophy.

Porous titanium implants with lower mechanical stiffness and better osseointegration have received increasing attention in recent years. Powder metallurgy techniques have been employed to produce porous titanium implants with varying pore size and interconnectivity (de Vasconcellos *et al.* 2012, Li *et al.* 2005, Pilliar 1983, Pinto Faria *et al.* 2010, Reis de Vasconcellos *et al.* 2008). However, bones have inhomogeneous, anisotropic structure, an example being long bones where the bone at the end exhibits a sponge-like structure (cancellous or trabecular bone), while the middle of the bone shaft is relatively dense (cortical bone). It would thus be desirable to design and fabricate titanium implants with a biomimetic hierarchical structure. Among various fabrication techniques, freeze casting is promising to produce such structures.

Freeze casting is a technique that produces porous materials by controlling the anisotropic solidification of a solvent, typically water, as a template within a powder suspension or sol-gel solution, followed by sublimation and solidification *via* sintering (Deville 2008, Wegst *et al.* 2010). Freeze casting has been primarily studied for porous ceramic production from a fine ceramic powder suspension. By subjecting the aqueous ceramic suspension to a temperature gradient, ice crystals will nucleate on one side of the suspension and grow along the temperature gradient. The ice crystals will redistribute the suspended ceramic particles as they grow within the suspension, effectively templating the porous ceramic. Chino and Dunand (Chino and Dunand 2008) and Li and Dunand (Li and Dunand 2011) first reported freeze cast titanium foams with aligned elongated pores using a water/agar suspension. They encountered several technical difficulties including large titanium particle size, high density and oxygen contamination. Incomplete sintering was also observed, which affected the pore structure and mechanical properties of resultant titanium foams. As an alternative, a reverse freeze casting technique has been developed, where camphene dendrites initially form a template, before the titanium slurry is introduced in order to overcome rapid sedimentation of the large and heavy titanium powders (Yook *et al.* 2012). However, this is an indirect process which require processing times exceeding 24 h.

In this work, we have used the freeze casting technique to form porous titanium alloy scaffolds with the use of a new fine Ti-6Al-4V powder and gelatin as a binder. The gelatin-gelation-freezing route has been reported to produce macroporous ceramics with honeycomb-like and non-dendritic pore structures (Fukushima *et al.* 2014). The rational of using gelatin as a binder in the titanium alloy slurries is to alleviate the sedimentation problem of coarse and dense titanium alloy particles during the freeze casting process, with the physically crosslinked gelatin able to hold the particles from fast sedimentation. Through the control of processing conditions, we aim at developing bone-like anisotropic and graded porosity structures for potential orthopaedic implant application.

2. Materials and Methods

2.1 Scaffold fabrication

Ti-6Al-4V powder (GR5 0-25 spherical powder, Advanced Powder & Coatings Ltd., Montreal, Canada) with a particle size of <25 μm was used as a raw material. 15 vol% Ti-6Al-4V powder was ball milled with 0.2 wt% dispersant (DOLAPIX CE 64, Zschimmer & Schwarz GmbH KG Chemische Fabriken, Lahnstein, Germany) and 1.5 wt% gelatin (G2500, Sigma-Aldrich, St. Louis, USA) in deionized water solution at 400 rpm for 4 h. The ball milled slurry was then placed in an oven at 60 °C for 20 min to dissolve the gelatin binder in water. Finally the slurry was ball milled at 80 rpm for an additional 12 h with 0.2 wt% octanol (General purpose grade, Fisher Scientific, UK) as a de-gassing agent. The bubble-free slurry was poured into a 60 mm diameter mould consisting of a stainless steel base and a PTFE jacket, and kept in a fridge at 4 °C to form a stable gel slurry. After gelation, the slurry within the mould was subject to unidirectional freezing in a custom built cooling apparatus (Preiss *et al.* 2012). The freeze casting temperature of the cooling side (bottom) was set at either -10, -15 or -20 °C. The temperature at the top of the slurry was kept at 20 °C. After freeze casting, the frozen slurry was sublimated under vacuum with a freeze drier (Modulyo, Edwards, UK) for 24 h. The dried bodies were debindered at 600 °C for 30 min before sintering at 1250 °C for 2 h under an argon atmosphere.

2.2 Scaffold characterisation

2.2.1 Density and porosity determination

30x15x5 mm samples were cut using a Struers Accutom-5 cutting machine. The specimens were divided into three layers (1.66 mm) in order to take into account the difference of porosity between the bottom, middle and top of the specimens. Archimedes' method was used to measure the density and the porosity of the cut specimens. The samples were first cleaned in an ultrasonic bath (Grant ultrasonic cleaner) and dried for 10 h in a desiccator. The dry samples were weighed (Satorius MC1 Analytic AC 210 S balance), then dipped into deionised water, boiled for 5 h and cooled in a fridge for 24 h (Bengisu 2001) to remove the remaining bubbles of air inside the pores. The samples were then suspended into water with a Satorius density determination kit to be weighed, and finally the weight of the wet sample, no longer suspended in water, was measured. The bulk density and the apparent porosity was calculated using the formulas:

$$\rho = \frac{D}{W - S} \tag{1}$$

$$P = 100 \times \frac{W - D}{W - S} \tag{2}$$

Where ρ is the density in g cm^{-3}, P the porosity percentage, D the weight of the wet sample in g, S the weight of the suspended sample in g, and W is the weight of the wet sample in g.

2.3 Microscopy

Cross-sections of each scaffold were cut from bottom to top and polished using #1200 and #2400 SiC paper. The samples were imaged using a Nikon SMZ-U stereo microscope with a Nikon D60 digital camera used for image capturing. Individual images were stitched together to form single images of the entire scaffold height. A JEOL IT 300 SEM was used for scanning electron microscope (SEM) imaging.

2.4 Micro Computed Tomography (microCT)

The microCT measurements were conducted on a Bruker SkyScan 1172 with a scan resolution of 10 μm. Image analysis was conducted using ImageJ (http://imagej.nih.gov/ij/). Porosity and pore sizes were calculated using the BoneJ plug-in. Drishti (https://sf.anu.edu.au/Vizlab/drishti/) was used to create the 3D image from the microCT data. An image stack using every fifth image from bottom to top of the sample was used for both image analysis and the 3D render.

2.5 Mechanical properties

Compressive testing was carried out to evaluate the yield strength. Six samples were cut from the top, middle and bottom of the original sample. The individual samples were then ground with a Struers TegraPol-15 polishing machine using #1200 SiC paper, to dimensions of 5x5x5 mm^3. Three samples from each layer were tested for compressive strength with the load parallel to the lamellar pores, and three samples were tested for compressive strength with the load perpendicular to the lamellar pores. A Zwick/Roell Z020 universal testing machine was used for the tests with the cross-head speed set at 0.5 mm min^{-1}.

3. Results and Discussion

3.1 Density and porosity of titanium alloy scaffolds

The titanium alloy slurries were freeze cast with three different temperatures applied at the bottom of the mould. To visualize the pores inside the scaffolds, the as-sintered samples were cut longitudinal from top to bottom and viewed with optical microscopy (Fig. 1), and transverse near the top and bottom of the samples and viewed with SEM (Fig. 2). Sedimentation of the titanium alloy particles was not observed for any of the freezing temperatures and the higher magnification SEM image in Fig. 3 showed that the titanium alloy powders were fully sintered.

Fig. 1 clearly show the effect of different freezing temperatures on pore sizes and gradient structure in the titanium alloy scaffolds. The samples displayed a gradient porous structure with lamella type pores running along the freezing direction. This was most evident for the -15 °C sample which clearly displayed anisotropic pores, aligned in the freezing direction. As expected with the unidirectional freeze casting, a lower bottom plate temperature, and thus faster freezing velocity, resulted in smaller pore sizes. The bottom section remained the densest for all the three samples, with increasing porosity further from the bottom freezing plate. From the transverse cross-sections in Fig. 2 it can be seen that the pores are more honeycomb-like than the plate-like pore structures typically seen for freeze cast slurries (Li and Dunand 2011). It is believed that the gelatin gelation used here helped create these more honey-comb like pores (Fukushima *et al.* 2014). The lamellar pores also had more connections than typically seen using other binders, e.g. polyvinyl alcohol (PVA) (Preiss *et al.* 2012), which may have potential implications to their mechanical properties, since a connected porous structure typically results in increased strength.

The pore sizes, as measured from the SEM images, at the top of the scaffolds were in the range of 10-80 μm for the -20 °C sample, 80-380 μm for the -15 °C sample and 150-430 μm for the -10 °C sample. Pore sizes of 100μm have been reported to be favourable for bone tissue ingrowth (Prananingrum *et al.* 2016), and the scaffolds produced here are thus of suitable dimensions for bone ingrowth, and can moreover be tuned for precise control of dimensions.

The apparent porosity in three different regions of each scaffold as measured by Archimedes' method is shown in Table 1. This data further confirmed the porosity gradient of the scaffolds.

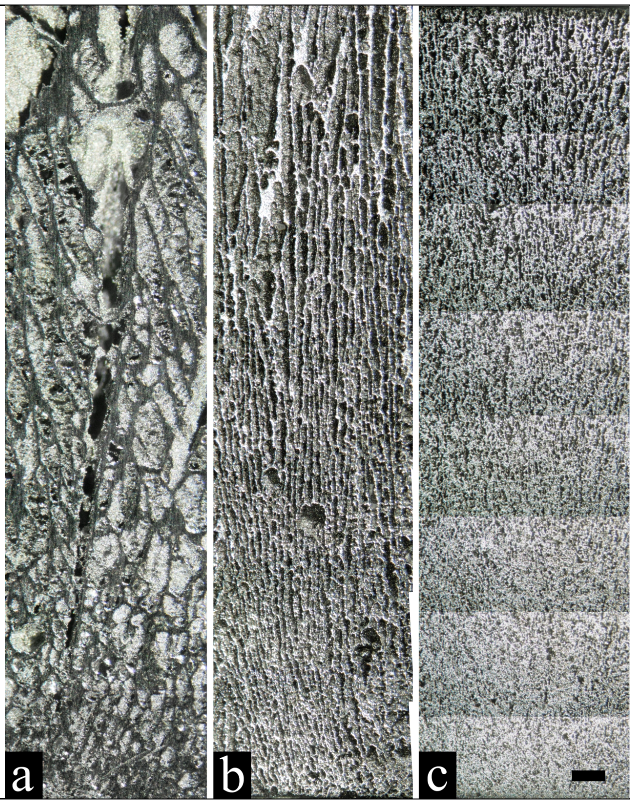

Fig. 1. Optical micrographs of freeze cast titanium alloy scaffolds at bottom freezing temperature of (a) -10 °C; (b) -15 °C; and (c) -20 °C, respectively. The scaffolds show a porosity gradient along freezing direction, with denser layer at the bottom and more porous layer at the top. The scale bar is 500 μm.

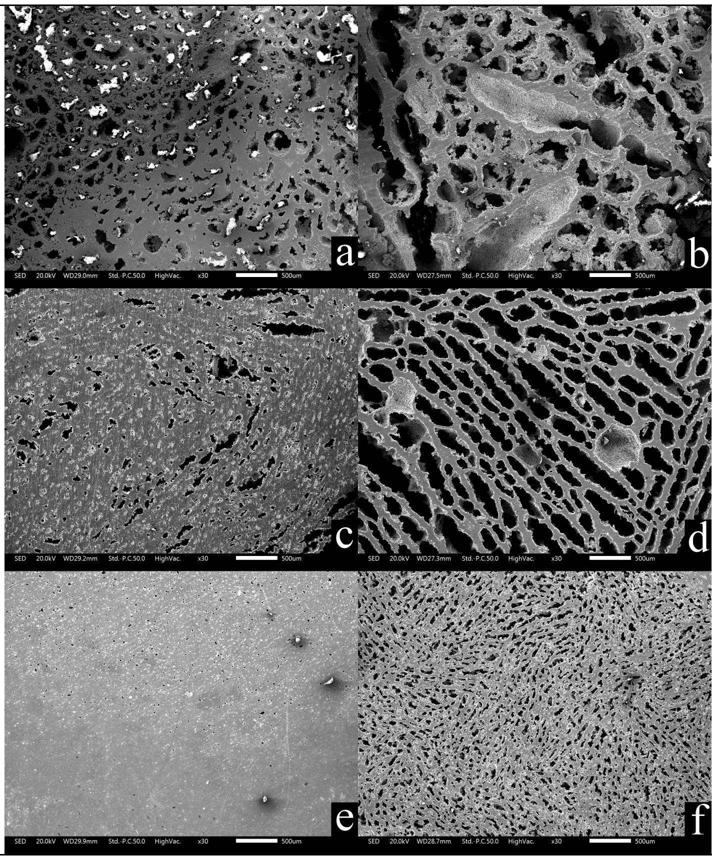

Fig. 2. Low magnification SEM images showing cross-sections of the samples at the bottom (a,c,e) and top (b,d,f) of the scaffolds. The image shows the pore morphology with the cross-section cut perpendicular to the lamellar pores. Bottom temperature -10 °C (a,b), -15 °C (c,d) and -20 °C (e,f).

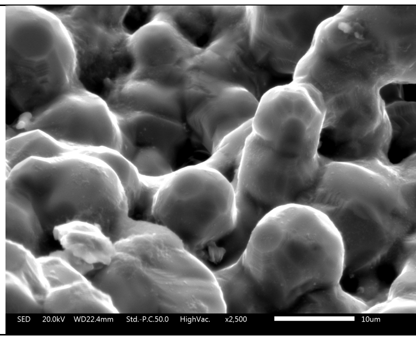

Fig. 3. SEM image showing fully sintered titanium alloy particles inside of a pore.

Table 1. The apparent porosity as measured by the Archimedes' method in the different regions of the samples freeze cast with different bottom plate temperatures.

	-10 °C	**-15 °C**	**-20 °C**
Porosity top	75.2 %	74.7 %	67.2%
Porosity middle	74.8 %	72.8 %	58.3%
Porosity bottom	73.7 %	69.9 %	49.9 %

The -15 °C sample was chosen for further characterization using microCT. A section of the scaffold, approximately 5x5 mm wide and with the full height of the scaffold was scanned to get an image of how the scaffold structure change with distance from the bottom freezing plate. Fig. 4 shows a rendered image of the scanned scaffold. Just as in the optical microscopy results, it is clearly seen that the scaffold exhibits a graded porous structure. Fig. 5 shows the porosity and the average pore size along the height of the sample (along the lamellar pores), as calculated from the microCT data. The average pore fraction increased from 40 % close to the freezing plate to 78 % on the top, while the average pore size increased from 40 µm to 155 µm. Again, the pore dimensions at the top of the scaffold are in the correct range for bone ingrowth. Previous methods have used powder metallurgy to create a porous surface on top of a dense titanium cylinder (de Vasconcellos *et al.* 2012). It is worth noting that the freeze casting technique presented here creates a complete gradient from dense to porous with the ability to control the porosity within the scaffold.

Fig. 4. MicroCT 3D rendered image of a titanium alloy scaffold freeze cast with a bottom plate temperature of -15 °C.

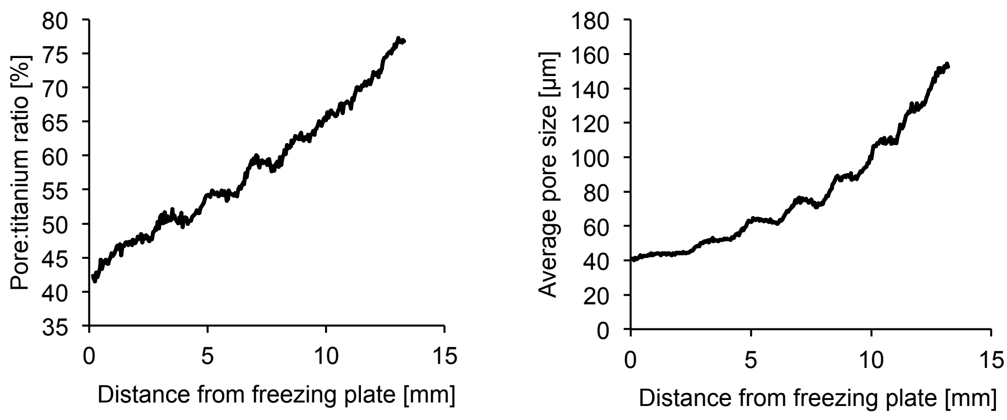

Fig. 5. Data retrieved from the microCT scan in Fig. 4. a) The average area ratio of pore:titanium in the scaffold, calculated in x-y slices at 50 µm intervals along the vertical axis (bottom to top of scaffold). b) The average pore size in each x-y layer at every 50 µm progressing along the vertical axis.

3.2 Mechanical properties of titanium alloy scaffolds

The compressive strength of titanium alloy scaffolds as a function of porosity is summarised in Fig. 6. Because the scaffolds were designed to have different mechanical properties at different heights of the scaffolds, the scaffolds were cut into six segments from bottom to top with the mechanical strength of each segment tested individually. The strength of the scaffolds decreased when moving from the bottom part towards the top, i.e. with increased porosity of the scaffold. The densest part of the scaffolds had a strength of up to 450 MPa, for samples fabricated with a bottom temperature of -20 °C. Crucially, not only does Fig. 6 demonstrate that there is a relation between porosity and strength independently of fabrication temperature, but also a clear gradient in strength within the individual scaffolds fabricated with different freezing temperatures. By controlling the freezing temperature during freeze casting it is thus possible to create a scaffold with varying mechanical properties along its z-axis. The strength of the scaffold segments was further dependent on whether the force was applied parallel or perpendicular to the pore direction. A greater strength was observed when the load was applied in parallel to the freezing direction, i.e. top to bottom of the scaffold, as opposed to when the load was applied perpendicular to the pores. This means that the scaffolds resemble the anisotropic structure of bone that is often stronger in the direction with higher mechanical load.

From the results in Fig. 6 it can be seen that it is possible to achieve compressive strength similar to that of cortical bone (200 MPa) (Oehman *et al.* 2011), with a porosity of about 60% along the freezing direction. Therefore, titanium alloy scaffolds with both bone-mimicking pore structure and mechanical properties could be produced for load-bearing applications in the repair or regeneration of damaged or diseased bones. The elastic moduli of titanium alloy scaffolds, estimated from compressive stress-strain curves, were in the range of 610 MPa and 2.3 GPa, which is close to the elastic modulus of cancellous bone but much lower than the dense titanium alloy (~110 GPa). This would greatly reduce the stress-shielding problem encountered in the current dense titanium metal and its alloy implants.

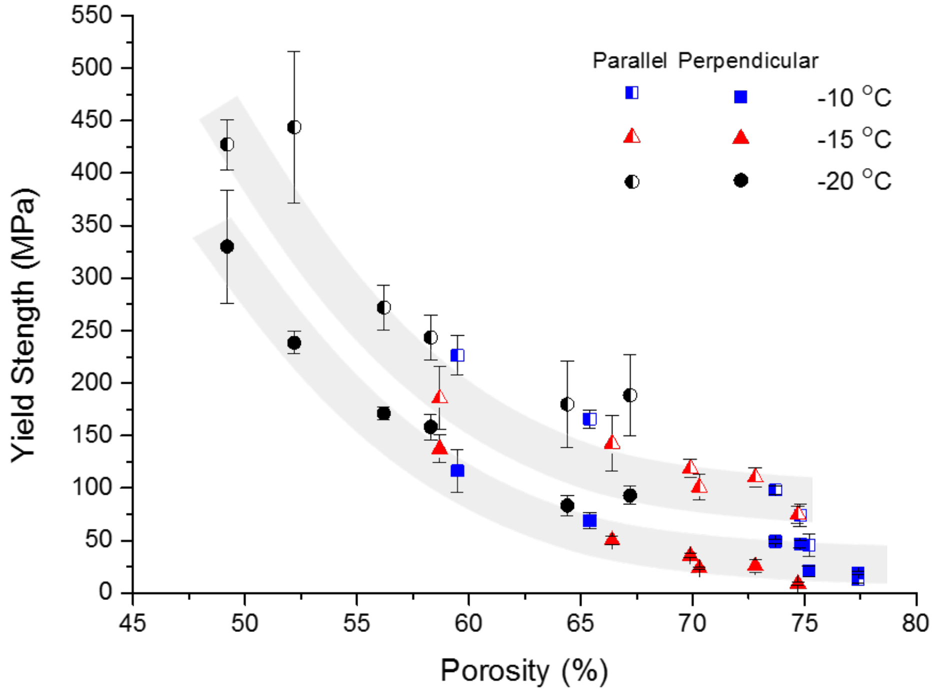

Fig. 6. Compressive strength with average porosity for all titanium alloy scaffolds freeze cast at different cooling temperatures. The hollow and solid symbols represent compressive strengths parallel and perpendicular to the freezing direction, respectively. The porosity of the tested samples was measured using Archimedes' method.

4. Conclusions

Biomimetic titanium alloy scaffolds with anisotropic and graded structure were produced by a unidirectional freeze casting technique using gelatin as a binder.

- By controlling the freezing temperature, graded porous scaffolds with aligned microstructure and connectivity were achieved.
- Pore dimensions at the porous end of the scaffolds were in the correct range to allow for bone ingrowth.
- Mechanical properties were anisotropic with a higher compressive strength in the freezing direction, similarly to the anisotropic nature of bone tissue.
- The scaffolds fabricated here with high mechanical strength (>100 MPa), low elastic moduli (<2.3 GPa) and graded porosities (from 50 - 70%), could be ideal candidates as load-bearing materials for orthopaedic and dental implant applications.

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